

IN THE CLAIMS:

The claims are as follows:

1. (Previously Presented) A nucleic acid molecule encoding a human Rhesus D antigen contributing to or indicative of the weak D phenotype, said nucleic acid molecule carrying at least one missense mutation, as compared to the wild type Rhesus D antigen set forth as SEQ ID NO:41, in its transmembrane and/or intracellular regions.
2. (Previously Presented) A nucleic acid molecule encoding a human Rhesus D antigen contributing to or indicative of the weak D phenotype, said nucleic acid molecule
 - a) carrying at least one missense mutation, as compared to the wild type Rhesus D antigen set forth as SEQ ID NO:41, in amino acid positions 2-16, 114-149, 179-225 or/and 267 to 397 with the proviso that said D antigen does carry not a single missense mutation leading to a substitution of phenylalanine in amino acid position 223 by valine or of threonine in position 283 by isoleucine; or
 - b) carrying a gene conversion involving exons 6 to 9 which are replaced by the corresponding exons of the RHCE gene.
3. (Previously Presented) The nucleic acid molecule of claim 1 or 2 wherein said missense mutation causes an amino acid substitution in position 3, 10, 16, 114, 149, 182, 198, 201, 220, 223, 270, 276, 277, 282, 294, 295, 307, 339, 385 or 393 or a combination of said substitutions.
4. (Previously Presented) The nucleic acid molecule of claim 3 wherein said amino acid substitution in position 3 is from Ser to Cys, in position 10 from Arg to Gln, in position 16 from Trp to Cys, in position 114 from Arg to Trp, in position 149 from Ala to Asp, in position 182 from Ser to Thr, in position 198 from Lys to Asn, in position 201 from Thr to Arg, in position 220 from Trp to Arg, in position 223 from Phe to Val, in position 270 from Val to Gly, in position 276 from Ala to Pro, in position 277 from Gly to Glu, in position 282 from Gly to Asp, in position 294 from Ala to Pro, in position 295 from Met to Ile, in position 307 from Gly to Arg, in position 339 from Gly to Glu, in position 385 from Gly to Ala [and] or in position 393 from Trp to Arg.

5. (Previously Presented) The nucleic acid molecule of any one of claims 1 or 2 wherein said missense mutation occurs in nucleotide position 8, 29, 48, 340, 446, 544, 594, 602, 658, 667, 809, 819, 826, 830, 845, 880, 885, 919, 1016, 1154 or 1177 or in a combination of said positions.
6. (Previously Presented) The nucleic acid molecule of claim 5 wherein said missense mutation in position 8 is from C to G, in position 29 from G to A, in position 48 from G to C, in position 340 from C to T, in position 446 from C to A, in position 544 from T to A, in position 594 from A to T, in position 602 from C to G, in position 658 from T to C, in position 667 from T to G, in position 809 from T to G, in position 819 from G to A, in position 826 from G to C, in position 830 from G to A, in position 845 from G to A, in position 880 from G to C, in position 885 from G to T, in position 919 from G to A, in position 1016 from G to A, in position 1154 from G to C and in position 1177 from T to C.
7. (Previously Presented) The nucleic acid molecule of claim 3 wherein said combination of substitutions is in positions 182, 198 and 201 or in position 201 and 223, or in position 16, 201 and 223.
8. (Previously Presented) The nucleic acid molecule of claim 5 wherein said combination of missense mutations comprises positions 544, 594 and 602 and is T to A at position 544, A to T at position 594 and C to G at position 602 or comprises positions 602, 677 and 819 and is C to G at position 602, T to G at position 667 and G to A at position 819 or comprises positions 48, 602, 667 and 819 and is G to C at position 48, C to G at position 602, T to G at position 667 and G to A at position 819.
9. (Previously Presented) The nucleic acid molecule of any one of claims 1 or 2 wherein said molecule is mRNA or genomic DNA.

10. (Previously Presented) A vector comprising the nucleic acid molecule of any one of claims 1 or 2.
11. (Previously Presented) A host cell transformed with the vector of claim 10.
12. (Previously Presented) A method of producing a Rhesus D antigen contributing to the weak D phenotype comprising culturing the host cell of claim 11 under suitable conditions and isolating the Rhesus D antigen produced.
13. (Withdrawn) A Rhesus D antigen encoded by the nucleic acid molecule of any one of claims 1 to 9 or produced by the method of claim 12.
14. (Previously Presented) An oligonucleotide hybridizing under 0.1X SSC, 0.1% SDS at 65⁰ C hybridization and washing conditions to a portion of the nucleic acid molecule of any one of claims 1 or 2 comprising said at least one missense mutation or to the complementary portion thereof or hybridizing to a region involving the breakpoint of the gene conversion identified in claim 2.
15. (Withdrawn) An antibody or aptamer or phage specifically binding to the Rhesus D antigen of claim 13.
16. (Withdrawn) An antibody or aptamer or phage specifically binding to the wild-type Rhesus D antigen or to aberrant Rhesus D antigens but not to the Rhesus D antigen of claim 13.
17. (Withdrawn) A method for testing for the presence of a nucleic acid molecule encoding a Rhesus D antigen contributing to the weak D phenotype in a sample comprising hybridizing the oligonucleotide of claim 14 under stringent conditions to nucleic acid molecules comprised in the sample obtained from human and detecting said hybridization.

18. (Withdrawn) The method of claim 17 further comprising digesting the product of said hybridization with a restriction endonuclease and analyzing the product of said digestion.
19. (Withdrawn) A method of testing for the presence of a nucleic acid encoding a Rhesus D antigen contributing to or indicative of the weak D phenotype in a sample comprising determining the nucleic acid sequence of at least a portion of the nucleic acid molecule of any one of claims 1 to 9, said portion encoding at least one of said missense mutations or a region involving the breakpoint of said gene conversion or of a nucleic acid molecule encoding a Rhesus D antigen contributing to the weak D phenotype, said nucleic acid molecule carrying a combination of missense mutations, as compared to the wild type Rhesus D antigen, one of said missense mutation causing an amino acid substitution in position 223 or 283 which is in position 223 preferably from Phe to Val and in position 283 preferably from Thr to Ile, said missense mutation further preferably occurring in nucleotide position 667 or 848 wherein most preferably said mutation in position 667 is form T to G and in position 848 from C to T.
20. (Withdrawn) The method of claim 19 further comprising, prior to determining said nucleic acid sequence, amplification of at least said portion of said nucleic acid molecule.
21. (Withdrawn) A method for testing for the presence of a nucleic acid molecule encoding a Rhesus D antigen contributing to or indicative of the weak D phenotype in a sample comprising carrying out an amplification reaction wherein at least one of the primers employed in said amplification reaction is the oligonucleotide of claim 14, comprising assaying for an amplification product.
22. (Withdrawn) The method of claim 20 or 21 wherein said amplification is effected by or said amplification is the polymerase chain reaction (PCR).
23. (Withdrawn) A method for testing for the presence of a Rhesus D antigen contributing to or indicative of the weak D phenotype in a sample comprising assaying a sample

obtained from a human for specific binding to the antibody or aptamer or phage of claim 15.

24. (Withdrawn) A method of testing a sample for the presence of wild type Rhesus D antigen and the absence of the Rhesus D antigen of claim 13 comprising assaying a sample obtained from a human for specific binding to an antibody or aptamer or phage of claim 16.
25. (Withdrawn) The method of any one of claims 17 to 24 wherein said sample is blood, serum, plasma, fetal tissue, saliva, urine, mucosal tissue, mucus, vaginal tissue, fetal tissue obtained from the vagina, skin, hair, hair follicle or another human tissue.
26. (Withdrawn) The method of 25 comprising enrichment of fetal cells or extraction of fetal DNA or mRNA from material tissue, like peripheral blood, serum or plasma.
27. (Withdrawn) The method of any one of claims 17 to 26 wherein said nucleic acid molecule or proteinaceous material from said sample is fixed to a solid support.
28. (Withdrawn) The method of claim 27 wherein said solid support is a chip.
29. (Withdrawn) Use of the nucleic acid molecule of any one of claims 1 to 9 or of a nucleic acid molecule encoding a Rhesus D antigen contributing to or indicative of the weak D phenotype, said nucleic acid molecule carrying a combination of missense mutations, as compared to the wild type Rhesus D antigen, one of said missense mutations causing an amino acid substitution in position 223 or 283 which is in position 223 preferably from Phe to Val and in position 283 preferably from Thr to Ile, said missense mutation further preferably occurring in nucleotide position 667 or 848 wherein most preferably said mutation in position 667 is from T to G and in position 848 from C to T or of a combination thereof for the analysis of a weak Rhesus D phenotype.

30. (Withdrawn) Use of the nucleic acid molecule of any one of claims 1 to 9, the vector of claim 10 or the Rhesus D antigen of claim 13 for the assessment of the affinity, avidity and/or reactivity of monoclonal antibodies or of polyclonal antisera preferably anti-D antisera, anti-globulin or anti-human-globulin antisera.
31. (Withdrawn) A method for the characterization of the monoclonal antibodies or polyclonal antisera or of a preparation thereof said method comprising
- (a) testing the nucleic acid of sample of a proband for the presence of a mutation as defined in any one of claims 1 to 8;
 - (b) correlating, on the basis of the mutation status and the allelic status of the RHD gene, the nucleic acid with the RhD antigen density on the surface of red blood cells of said proband;
 - (c) reacting said monoclonal antibodies or polyclonal antisera or said preparation thereof with a cell carrying the RhD antigen on its surface;
 - (d) characterizing said monoclonal antibodies or polyclonal antisera or said preparation thereof on the basis of the results obtained in step (c).
32. (Withdrawn) The method of claim 31 wherein said characterization comprises the determination of reactivity, sensitivity, avidity, affinity, specificity and/or other characteristics of antibodies and antisera.
33. (Withdrawn) The method of claim 31 or 32 wherein said cell carrying the RhD antigen on its surface is a red blood cell.
34. (Withdrawn) Use of an aptamer, phage, monoclonal antibody or a polyclonal antisera as characterized in claims 15 or 16 or a preparation thereof for RhD antigen determination.
35. (Withdrawn) Use of claim 34 wherein said RhD antigen determination is effected in connection with blood group typing.

36. (Withdrawn) A preparation comprising the antibody or aptamer or phage of claims 15 or 16.
37. (Withdrawn) A method of identifying an antibody V_H or V_L chain or a combination thereof or an aptamer specifically binding to a weak D antigen of claim 13 comprising
- (a) contacting the weak D antigen of claim 13 with a phage library displaying V_H or V_L chains or combinations thereof on the surface of the phage or with aptamers;
 - (b) identifying phage or aptamers that bind to said weak D antigen; and optionally
 - (c) repeating steps (a) and (b) one or more times.
38. (Withdrawn) A method of identifying a monoclonal antibody specifically binding to a weak D polypeptide/antigen of claim 13 comprising
- (a) contacting the weak D polypeptide of claim 13 with one or more monoclonal antibodies;
 - (b) identifying monoclonal antibodies that bind to said weak D polypeptide; and optionally
 - (c) repeating steps (a) and (b) one or more times.
39. (Withdrawn) A method of identifying an antibody V_H or V_L chain or a combination thereof or an aptamer specifically binding to a weak D polypeptide/antigen of claim 13 comprising
- (a) contacting the weak D polypeptide and
 - (aa) a second or more weak D polypeptide(s) and/or
 - (ab) a normal D polypeptidewherein the second or more weak D polypeptide(s) and/or the normal D polypeptide are present in a molar mass that is higher, equal or less than the weak D polypeptide of (a) with a phage library displaying V_H or V_L chains or combinations thereof on the surface of the phage or with aptamers;
 - (b) identifying phage or aptamers that bind to said weak D polypeptide of (a); and optionally
 - (c) repeating steps (a) and (b) one or more times.

40. (Withdrawn) A method of identifying a monoclonal antibody specifically binding to a weak D polypeptide/antigen of claim 13 comprising
- (a) contacting the weak D polypeptide and
 - (aa) a second or more weak D polypeptide(s) and/or
 - (ab) a normal D polypeptide
- wherein the second or more weak D polypeptide(s) and/or the normal D polypeptide are present in a molar mass which is higher, equal or less than the weak D polypeptide of (a) with one or more monoclonal antibodies;
- (b) identifying monoclonal antibodies that bind to said weak D polypeptide of (a); and optionally
 - (c) repeating steps (a) and (b) one or more times.
41. (Withdrawn) The method according to any one of claims 37 to 40, wherein the weak D polypeptide is exposed on the surface of a cell.
42. (Withdrawn) The method according to any one of claims 37 to 41, wherein the polypeptide or host cell is affixed to a solid support.
43. (Withdrawn) The method of any one of claims 37 to 42, wherein subsequent to step (b) or (c), the following step is carried out:
- (d) identifying the amino acid sequence of the V_H or V_L chains and/or identifying the nucleic acid sequence encoding said amino acid sequence.
44. (Withdrawn) The method according to any one of claims 41 to 43, wherein, in the case that only one round of selection is employed for the identification, the number of weak D polypeptide molecules of (a) is in molar excess over the number of phage particles.
45. (Withdrawn) Use of cells, preferably red blood cells, restricting to cells comprising the RhD-antigen of claim 13 from probands for the assessment of the affinity, avidity and/or reactivity of monoclonal anti-D antibodies or of polyclonal anti-D antisera or of anti-globulin or of anti-human-globulin antisera or of preparations thereof.

46. (Withdrawn) A method for determining whether a patient in need of a blood transfusion is to be transfused with RhD negative blood from a donor comprising the step of testing a sample from said patient for the presence of one or more RhD antigens of claim 13, wherein a positive testing for at least one of said antigens is indicative of the need for a transfusion with RhD negative blood.
47. (Withdrawn) A method for determining whether blood of a donor may be used for transfusion to a patient in need thereof comprising the step of testing a sample from said donor for the presence of one or more RhD antigens of claim 13, wherein a positive testing for at least one or said antigens excludes the transfusion of patients that are typed as having wild type Rh D antigen or (a) weak D type(s) other than the weak D type(s) of said donor.
48. (Previously Presented) Kit comprising the oligonucleotide of claim 14.
49. (Previously Presented) The nucleic acid molecule of claim 7, wherein said combination of substitutions in positions 182 is from S to T, 198 from K to N, and 201 from T to R; positions 201 is from T to R and 223 from F to V; or in positions 16 is from W to C, 201 from T to R, and 223 from F to V.
50. (Previously Presented) The oligonucleotide of claim 14, said oligonucleotide 12 to 50 nucleotides in length.
51. (Previously Presented) The oligonucleotide of claim 14, said oligonucleotide 15 to 24 nucleotides in length.